Use of Starter Cultures in Meats

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ABSTRACT

Use of starter cultures in meat products is reviewed, with emphasis on the types of microorganisms employed for production of various products, and the effect of starter cultures on food safety. Desirable starter culture characteristics are identified, and the effect of fermentation on the nutritive quality of meats is considered. Food safety aspects of starter culture use discussed include the effects on survival of viruses, trichinae, and pathogenic bacteria, and on the control of mycotoxin, nitrosamine, and presumor amine contamination.

The dairy industry has had a long history of preparing fermented products with starter cultures containing known microorganisms with proven metabolic activity. However, less than 50% of meat processors use starter cultures to produce fermented sausages (*6*), although it is probable that on a volume basis, more fermented sausages are produced with starter culture than without. Many manufacturers of fermented sausages still use natural inoculation techniques such as "back-slopping" (adding meat reserved from a previous successful fermentation to the sausage mix) or enriching for fermentative microorganisms by aging salted meats at low temperatures. Both techniques can be successful as long as the desired microbial types are the predominant flora. However, failures may occur if nonfermentative microorganisms or heterofermentative lactic acid bacteria predominate. Addition of large numbers (10^7 to 10^9/g) of desirable microorganisms will inhibit growth of undesirable species, thereby preventing or reducing fermentation failures. In this review, the focus is on microorganisms used as starter cultures in fermented meat products and on contributions of starter culture organisms to food safety. Other aspects concerning the use of starter cultures are reviewed by Coretti (*23*), Bacus and Brown (*9*), Smith and Palumbo (*80*), and Haymon (*39*).

CHARACTERISTICS OF MEAT STARTER CULTURES

A meat starter culture can be defined as viable microorganisms added directly to meat to improve the keeping quality, improve the safety, and/or enhance consumer acceptability of the meat product. In addition, the nutritional quality of the meat should be maintained or improved. Deibel (*27*) discussed characteristics that are desirable in a meat starter culture. The microorganisms (*a*) should be salt- and nitrite-tolerant (grow vigorously at 6% NaCl and 100 ppm nitrite), (*b*) must grow at a temperature range of 27 to 43°C (80 to 110°F) with the optimum at approximately 32°C (90°F), (*c*) must not produce compounds associated with off-odors, and (*d*) must not be harmful to health (be neither pathogens nor produce toxic compounds as part of their metabolism). If the starter culture is a lactic acid microorganism, it must be homofermentative since gas production and fermentation products other than lactic acid contribute to off-flavors and other defects. Deibel (*27*) also suggested that the lactic acid bacteria should not be proteolytic or lipolytic; however, these reactions may be desirable in specific types of fermented sausages.

USES OF STARTER CULTURES IN MEATS

A variety of semi-dry and dry fermented sausages can be produced using single species of *Pediococcus cerevisiae*, *P. pentosaceum*, *Lactobacillus plantarum*, or *Penicillium* spp., or mixtures of *P. cerevisiae* and *L. plantarum* or *P. cerevisiae* and *Micrococcus varians* (Table 1). The major role of the lactic acid bacteria is to produce lactic acid rapidly and reliably from sugars (usually glucose) added to the sausage mixture. Lactic acid lowers the pH of fermented sausages, thereby enhancing the product's keeping qualities. Additionally, lactic acid imparts a tangy flavor to the product and denatures the meat protein. This denaturation, which also results in water expulsion, is largely responsible for the texture associated with fermented sausages. The role of the fungi in mold-ripened sausages (limited to the outer surface of the sausage) is to produce metabolic products that contribute to the taste, odor, and keeping qualities

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TABLE 1. *Microorganisms used as starter cultures in meat.*

Microorganism	Type of product (reference)
I. Bacteria	
1. *Pediococcus cerevisiae*	A. Semi-dry fermented sausages
	a. summer sausage (*3,4,26*)
	b. cervelat (*31,46*)
	c. Thuringer (*46*)
	d. pork roll (*57*)
	e. summer-style turkey sausage (*43*)
	B. Dry fermented sausages
	a. dry sausage (*52*)
	b. dry turkey sausage (*11*)
	c. salami (*53*)
	d. pepperoni (*53*)
	e. hot bar sausage (*46*)
	C. Processed meat
	a. country-style ham (*13,14*)
2. *Pediococcus pentosaceus*	A. Semi-dry fermented sausage
	a. summer sausage (*65*)
	B. Dry fermentaged sausages
	a. pepperoni (*65*)
	b. Genoa (*65*)
3. *Lactobacillus plantarum*	A. Semi-dry fermented sausage
	a. summer sausage (*54*)
	B. Dry fermented sausages
	a. salami (*42*)
	b. European-type dry sausage (*60*)
	C. Processed meat
	a. bacon (*84*)
	b. country-style ham (*14*)
4. *Lactobacillus brevis*	A. Fresh meat
	a. minced meat (*34*)
5. *Mixture of P. cerevisiae* and *L. plantarum*	A. Semi-dry fermented sausages
	a. lebanon bologna (*82,88*)
	b. summer sausage (*21,44*)
	c. cervelat (*42*)
	B. Dry fermented sausages
	a. pepperoni (*81*)
	b. dry turkey sausage (*2*)
	C. Processed meat
	a. cooked, mechanically deboned poultry meat (*68*)
	D. Fresh meat
	a. mechanically deboned poultry meat (*69*)
	b. ground poultry breast meat (*70*)
6. Mixture of *P. cerevisiae* and *Micrococcus varians*	A. Dry fermented sausage
	a. Genoa (*38*)
	b. dry sausage (*1*)
II. Fungi and yeast	
1. Individual *Penicillium* species: *P. janthinellum, P. simplicissimum, P. cyclopium* or *P. viridicatum*	A. Dry fermented sausage
	a. mold-ripened salami sausage (*22*)
2. *Thamnidium elegans*	A. Fresh meat
	a. beef carcass aging (*7*)
3. *Candida lipolytica*	A. Fresh fish
	a. fish (*17*)

of the sausages. Surface mold growth contributes to the appearance of the product and prevents growth of undesirable microorganisms (*64*).

A recently introduced starter culture for production of fermented sausages is the mixture of *P. cerevisiae* and *M. varians* (*38*). *P. cerevisiae* is a strong lactic acid producer, whereas *M. varians* is a poor acid producer. The micrococcus is added to improve the flavor and color of the sausages. Catalase produced by the micrococci decomposes peroxides produced in meats by microbial action or by oxidative reactions. Peroxides can lead to color defects. Raccach and Baker (*67*) have shown that *P. cerevisiae* and *L. plantarum* present in commercial meat starter cultures produce very little peroxides as H_2O_2.

Bartholomew and Blumer (*13,14*) suggested inoculation of hams with *P. cerevisiae* or *L. plantarum* to shorten the processing time necessary to produce country-style hams. Tanaka et al. (*84*), by inoculating bacon containing >0.5% sucrose with *L. plantarum*, could decrease the level of nitrite or eliminate the need for it completely. The acid produced by the lactobacillus prevented growth of *Clostridium botulinum*, and therefore less nitrite was needed.

An interesting aspect of starter culture use is in the extension of the shelf life of meats, particularly fresh meats. Fetlinski et al. (*34*) found that addition of a *Lactobacillus brevis* culture to minced beef inhibited growth of gram-negative microorganisms, resulting in a shelf life increase of serveral days. A 50/50 mixture of *P. cerevisiae* and *L. plantarum* extended the shelf life of both cooked and raw mechanically-deboned poultry meat as well as that of ground chicken by inhibiting growth of various spoilage *Pseudomonas* species (*68-70*). Other workers have shown that the shelf life of ground beef and beef steaks was prolonged by treatment with various lactic acid bacteria (*24,35,71,72*).

Smith et al. (*77*) and Egan and Shay (*30*) did not recommend the inoculation of vacuum-packed fresh beef with lactic acid bacteria due to potential off-odors, surface discoloration, and lowered palatability. Inoculation of fresh meats with lactic acid bacteria may be desirable from a microbiological point of view since they inhibit gram negative psychrotrophic spoilage microorganisms; however, their effect on meat quality and sensory characteristics may preclude their use. Moon et al. (*58*) have shown that while strains of *Streptococcus lactis* and *Lactobacillus casei* inhibit growth of the spoilage *Pseudomonas* species in culture media, they were not effective in preventing spoilage of shrimp during storage in ice. Use of lactic acid bacteria as agents in prolonging the shelf life of fresh meat products appears to be controversial and warrants further study to determine if they do have potential as anti-spoilage agents.

Normally, aging of meat occurs at low temperatures to control undesirable bacterial growth on the carcasses. Spraying beef carcasses with *Thamnidium elegans* prevented growth of bacteria and allowed tenderization by meat proteases to take place at elevated temperatures and humidities (*7*). Aging of the meat could be completed in 2 to 3 d at elevated temperatures (>25°C) with use of the mold as compared to the normal 2 to 3 weeks at 0°C; however, at >25°C, tenderization of the carcass will take place in the absence at the mold. Kotula (*48*) reported that the use of *T. elegans* on carcasses stored at 4°C did not tenderize the meat; further studies indicated that little or no proteolytic enzyme was produced by the mold at 4°C (*49*).

Mehadden, a variety of fish which is used for fish meal and oil but not for human food, can be rendered into a product acceptable for human consumption by fermentation with *Candida lipolytica* or other yeasts (*17*). The fermented fish product had increased protein and decreased fat levels and was neutral in flavor. Potentially, this fermentation procedure could be used to transform other "trash" fish into an acceptable food for humans.

NUTRITIONAL ASPECTS OF FERMENTED SAUSAGES

Information concerning the effect of fermentation on the nutritive value of meat is quite limited. Using *Lactobacillus-Micrococcus* and *Lactobacillus* starter cultures, respectively, Dierick et al. (*29*) and Eskeland and Nordal (*32*) reported that the level of free amino acids in European-style sausages increased during ripening. Eskeland and Nordal (*32*) found an increase in the digestibility of the meat proteins with fermentation and ripening. It was not clear whether the changes found in the dry sausages are the result of starter culture activity or other factors since these workers did not simultaneously study nonfermented sausages or sausages containing inactivated starters. However, Niinivaara et al. (*61*) concluded that the increased free amino acid level found in dry sausages was produced by the action of meat tissue enzymes, since the free amino acid pattern was similar in the sausages produced with or without a *Micrococcus* starter culture.

Lactic acid bacteria are rather fastidious and require preformed amino acids, B-vitamins, and purine and pyrimidine bases for growth (*41*); thus, in some respects, especially in vitamin and amino acid content, the nutritive value of meat may decrease on fermentation. By its preservative effect, fermentation contributes to nutrition because the product is available for consumption long after fresh or cooked meats have spoiled. Research is needed concerning the influence of fermentation on the nutritive quality of meat.

EFFECT OF STARTER CULTURES ON TRICHINAE

There appears to be no literature available dealing with the direct effect of starter cultures on trichinae viability. However, drying is an important process step that aids in the inactivation of trichinae in dried fermented (and uncooked) sausages. Bacus and Brown (*9*) suggested that microbial starter cultures may contribute indirectly to the destruction of trichinae, since controlled fermentation allows for a more consistent drying rate. The parasite is also adversely affected by decreased pH (*18*), and it is possible that lactic acid starter cultures, in the presence of fermentable sugars, aid in trichinae inactivation. Studying trichinae inactivation in Genoa salami, Childers et al. (*20*) indicate that the level of NaCl was the most important factor influencing destruction of trichinae. The Genoa salami was prepared using a mixed starter culture of *M. varians* and *P. cerevisiae* but Childers and his coworkers did not directly study the effect of the starter culture microorganisms on trichinae death. It would be of interest to determine if the lactic acid starter culture organisms have a major or minor role in the destruction of trichinae.

BIOGENIC PRESSOR AMINES AND MEAT STARTER CULTURES

One of the problems that processors involved in food fermentations must continually face is the possibility that the fermentative microflora may produce potentially toxic compounds from nontoxic constituents normally present in

the food. Baumgart et al. (*15*) found that *P. cerevisiae* produced histamine by decarboxylating histidine and *L. brevis* decarboxylated tyrosine or histidine to tyramine or histamine, respectively. Other lactic acid bacteria such as *L. plantarum, L. buchneri,* or *L. delbrueckii* did not produce these amines. Histidine and tyrosine decarboxylase levels were low or not detectable in *P. cerevisiae* (four strains including two commercial starter cultures). *L. plantarum* (three strains including two commercial starter cultures), and in one strain each of *L. acidophilus* and *L. casei* (*73*).

A variety of fermented dry and semi-dry sausages contain the pressor amines, tyramine, tryptamine, phenylethylamine, and histamine (*45,74,86*). In general, the dried sausages had higher concentrations of pressor amines than the semi-dry sausages. This is probably due to the longer aging period that dry sausages undergo, as well as to the concentrating effects of the drying process itself.

Eitenmiller et al. (*31*) have shown that higher levels of tyrosine decarboxylase were present during fermentation of cervelat when a natural fermentation technique (aging of salted ground meat at 5°C) was used, as compared to the sausages fermented by addition of a commercial starter culture containing *P. cerevisiae*. Since the commercial meat starter cultures lack amino acid decarboxylases necessary to produce the toxic amines (*73*), it would appear that the amines in fermented sausages were produced either by contaminating microorganisms or the microflora developed by natural fermentation enrichment techniques.

Presence of biogenic amines can be controlled in fermented sausages by rigid employment of good hygiene in both raw materials and manufacturing environments, and by the use of short fermentation times employing active starter cultures lacking amino acid decarboxylases. Use of starter cultures with known metabolic activity instead of natural fermentation techniques will do much to alleviate the build-up of potentially toxic amines in fermented sausages. Hopefully, as new starter cultures are developed, they will be screened for their ability to produce toxic amines.

NITROSAMINES AND LACTIC ACID STARTER CULTURES

Strains of *L. plantarum,* including those used in commercial meat starter cultures, reduce nitrate to nitrite (by the action of nitrate reductase) in both a nutrient medium and meat (*79*); a commercial *P. cerevisiae* strain lacked nitrate reductase. The ability of meat starter cultures containing *L. plantarum* to lower the pH of sausage meats containing a fermentable sugar, coupled with its nitrate reducing capacity, could create conditions that would lead to nitrosamine formation from secondary amines and nitrite. This would be expected to be particularly true in sausages formulated with high levels of nitrate. However, a survey which included a large number of dry and semi-dry fermented sausages, did not indicate detectable levels of volatile nitrosamines in these products (*8*). Nitrosamines were absent in Lebanon bologna prepared with a straight nitrate cure and in conjunction with either a mixed lactic acid starter culture (*P. cerevisiae* and *L. plantarum*) or a natural fermentation (*63*). Dethmers et al. (*28*), using a mixed starter culture (*P. cerevisiae* and *L. plantarum*) with various nitrite-nitrate cures (0 to 150 ppm nitrite; 0 to 1500 ppm nitrate), did not find volatile nitrosamines in Thuringer sausages. Nitrosamines were not detected in European-type dried sausages prepared with starter culture (*Lactobacillus-Micrococcus* mixture or *P. cerevisiae*) or natural fermentation (*47*). It is not clear whether the absence of nitrosamines in fermented sausages, even when the processing conditions appear to be favorable for their formation, is due to lack of nitrosamine formation or their subsequent breakdown. The role of the starter cultures, if any, is uncertain; however, nitrosamine formation may not be possible due to decomposition of nitrite by the lactic acid produced in fermented sausages.

N-nitrosopyrrolidine is formed in cooking bacon and the level of nitrosamine is directly related to the nitrite level (*37*). The concentration of residual nitrite in bacon can be reduced by using lactic acid starter cultures in the curing process (*33,36*). During smoking and subsequent processing of bacon bellies, the lactic acid bacteria grow and use the sugar present in the cure, thereby reducing the pH of the meat. The lowered pH aids in dissipating the nitrite. In addition, such acidulated bacon (*L. plantarum* plus >0.5% sucrose) does not support growth of *C. botulinum* upon temperature abuse (*84*) even though the nitrite content is low.

MYCOTOXINS AND MOLD STARTER CULTURES

In commercial practice, pure cultures are not used in the production of mold-ripened salamis (*22*). The predominant flora which develops on the casing is *Penicillium* or *Penicillium* and *Scopulariopsis* species (*16*). When Ciegler et al. (*22*) used pure cultures of various *Penicillium* species to inoculate the surface of sausages, no penicillic acid was detected (at 2, 4, and 10 weeks) even though the molds produced the toxin in vitro. Penicillic acid added to the sausage mix formed an adduct with sulfhydryl amino acids and thus escaped detection; the adduct was not toxic to mice and quail, but was toxic to chick embroyos.

Undesirable organisms developing on the surface of mold-ripened salamis are members of the genus *Aspergillus* (*16*). Bullerman and his coworkers incubated sausages at low temperatures (15°C or below) and low relative humidities (below 70%) to prevent growth and aflatoxin formation by *Aspergillus flavus*. Smoking the salamis also was an aid in decreasing aflatoxin formation. The level of aflatoxin produced by *A. flavus* in salamis incubated at 20°C and 75% to 80% humidity was low (1 to 3 μg/g) in contrast to the level produced on rice (330 to 480 μg/g) (*16*). Similarly, Strzelecki (*83*) found very low levels of aflatoxin metabolites in salamis inoculated with *A. flavus* and stored at a variety of temperatures. Therefore, meat does not appear to be a good substrate for aflatoxin production. However, Tauchman and Leistner (*85*) concluded that some strains of *A. flavus* or *Aspergillus parasiticus* can produce high concentrations of aflatoxins on a meat substrate.

While it may be questioned whether or not meat makes a good substrate for mycotoxin production, the industry nevertheless should be using pure culture techniques, i.e., using known fungi which have been screened for their lack mycotoxin synthesis in the production of mold-ripened salamis.

In fermented sausages surface-inoculated with *A. flavus*, Alvarex-Barrea et al. (*5*) showed that the starter culture organisms (a combination of *P. cerevisiae* and *M. varians*) were unable to prevent production of aflatoxin B_1 by the fungi. Smoking of the sausages combined with low temperature storage (10°C) under low humidities (75% to 79% relative humidity) did prevent formation of toxic fungal metabolites.

Murthy et al. (*59*) found that pigs fed peanut meal naturally contaminated with aflatoxins had low levels of fungal metabolites in liver, gall bladder, spleen, heart, muscle, and kidney. There do not appear to be any studies on the effect of lactic acid starter cultures on aflatoxins found in fermented sausages produced from meats obtained from animals fed aflatoxin-contaminated diets. However, data presented by Wiseman and Marth (*87*) indicates that lactic acid starter cultures used to produce yogurt, buttermilk, or kefir from milk naturally contaminated with aflatoxin M_1 did not decrease the mycotoxin level; thus, lactic acid starter cultures probably lack the capacity to degrade aflatoxins.

VIRUSES AND LACTIC ACID STARTER CULTURES (Table 2)

The fate of viruses present in meat used for fermented sausage fermentation has received little attention. The concentration of coxsackievirus decreased from 7.5×10^5/g to 1.1×10^3/g during the fermentation of contaminated Thuringer sausage (*40*). The starter organism, a *Lactobacillus* species, increased in number from 4.0×10^6/g to approximately 10^9/g with a concomitant decrease in pH from 6.02 to 4.8. Kantor and Potter (*42*) observed virtually no destruction of echovirus or poliovirus during starter culture fermentation (*L. plantarum* plus *P. cerevisiae*) and subsequent low temperature storage of both cervelat and dry fermented salami. The final pH of the sausages ranged from 5.0 to 5.5.

In pepperoni and dry fermented sausages with *P. cerevisiae* starter culture, McKercher et al. (*53*) showed that both African swine fever and hog cholera viruses were completely destroyed in these sausages by the end of the drying period; unfortunately, the final pH of the sausages was not given. The data presented by the three research groups indicated that quite variable destruction of virus results during sausage fermentation. The inconsistencies may depend on the type of virus studied but the amount of work done in this important area is so limited that no pattern is apparent.

FOODBORNE BACTERIA AND LACTIC ACID STARTER CULTURES

The undissociated lactic acid molecule is inhibitory to the growth of microorganisms (*41*). The main product of sugar fermentation by lactic acid bacteria is lactic acid; the ensuing low pH ensures that considerable amounts of unionized lactic acid (pK, 3.87) is available to prevent growth of undesirable microorganisms. Thus lactic acid bacteria contribute to microbial food safety in fermented meat products because most, if not all, food poisoning bacteria are susceptible to acidic conditions (*75*).

Salmonellae (Table 3)

Salmonella typhimurium and *Salmonella dublin* disappeared more rapidly during the fermentation of Lebanon bologna at 35°C using a mixed starter culture (*L. plantarum* plus *P. cerevisiae*) than when a natural flora fermentation procedure was used (aging of salted ground beef at 5°C for 10 d). However, at the end of the 4-d fermentation period, viable salmonellae were not present in the sausage regardless of the fermentation procedure; the final pH of the sausage was 4.0 to 4.1 (*82*). Salmonellae remained viable in nonfermented sausages (pH was 5.3 at 4 d). *S. dublin* was more resistant to the acid conditions than *S. typhimurium*. Smoking of the Lebanon bologna also contributed to destruction of salmonellae (*82*).

Pepperoni undergoes a shorter fermentation period at 35°C than Lebanon bologna so that neither *S. dublin* nor *S. typhimurium* was eliminated during sausage fermentation regardless of the fermentation method (1-d fermentation with starter culture or 2 d with natural flora fermentation). After a fermentation period, the pepperoni were then sub-

TABLE 2. *Investigations on the effect of meat starter cultures on the survival of viruses present in meat products.*

Foodborne pathogen (food)	Starter culture organisms	Reference
Coxsackievirus (Thuringer)	a *Lactobacillus* species	*40*
Echovirus (dry fermented salami)	*Lactobacillus plantarum*	*42*
Echovirus (cervelat)	*L. plantarum* plus *Pediococcus cerevisae*	*42*
Poliovirus (dry fermented salami)	*L. plantarum*	*42*
Poliovirus (cervelat)	*L. plantarum* plus *P. cerevisiae*	*53*
African swine fever virus (dry fermented salami)	*P. cerevisiae*	*53*
African swine fever virus (pepperoni)	*P. cerevisiae*	*53*
Hog cholera virus (dry fermented salami)	*P. cerevisiae*	*53*
Hog cholera virus (pepperoni)	*P. cerevisiae*	

jected to a drying period at temperatures and times specified for destruction of trichinae. The final pH of the fully dried starter culture-fermented pepperoni was 4.5 while that of the natural flora fermented was 5.0. Use of a mixed starter culture (*L. plantarum* plus *P. cerevisiae*) led to the complete destruction of *S. typhimurium* by the end of the 43-d drying period but *S. dublin* was still present (*81*). In natural flora-fermented pepperoni (aged, salted meat), low numbers of both *S. dublin* and *S. typhimurium* were still present at the end of 42 d of drying. There was no destruction of *S. dublin* in dried nonfermented pepperoni (final pH 5.7). Interestingly, destruction of *S. typhimurium* in nonfermented pepperoni was similar to that found in natural flora-fermented pepperoni (*81*), which suggests that drying alone was effective in eliminating *S. typhimurium*. Destruction of *S. dublin*, however, was achieved by a combination of acid conditions and drying. Complete destruction of salmonellae in pepperoni could only be assured by heating the sausages (after fermentation but before drying) to an internal temperature of 60°C.

Sirviö et al. (*76*) found that in the absence of starter culture, salmonellae grew in Rohwurst during the early stages of processing and drying, and viable salmonellae were present in the final product. However, in the presence of starter culture (*Lactobacillus* plus *Micrococcus*), no growth of *Salmonella senftenberg* (initial level of 10^4/g) was observed and the numbers of viable salmonellae decreased to undetectable levels within 7 to 14 d of processing. The pH of the product after 32 d of drying was 4.9. It is probable that, as with pepperoni the effectiveness of the starter culture in eliminating salmonellae in Rohwurst was due to acid production.

Viable *Salmonella pullorum* and *S. senftenberg* were found in dry fermented turkey sausage at the end of fermentation (starter culture was *P. cerevisiae*) and drying steps (*10*). Despite considerable destruction of salmonellae during the manufacture of the turkey sausage, complete elimination was not achieved.

In the manufacture of summer sausage, Masters et al. (*54*) observed that *Salmonella newport* and *S. typhimurium* were eliminated completely by use of a *L. plantarum* starter culture. The rapid reduction of pH brought about by the starter culture enhanced salmonellae destruction as well as shortened the processing time.

A 50/50 mixture of *L. plantarum* and *P. cerevisiae* (to give approximately 10^8 lactic acid bacteria/g) prevented growth of *S. typhimurium* present initially at 10^3/g in cooked mechanically deboned poultry meat stored at 11°C (*68*). At the end of 7 d of storage, the number of *S. typhimurium* was approximately 10^3/g in the lactic acid bacteria-treated meat, while the control meat had a salmonellae population of approximately 10^8/g. Growth inhibition was not related to acid production since the pH had decreased by only 0.2 to 0.3 unit.

An enteropathogenic strain of *Escherichia coli* was somewhat resistant to processing conditions (fermentation by *P. cerevisiae* followed by drying) used for the manufacture of dry fermented turkey sausage (*10*). With an initial population of approximately 10^6 *E. coli* cells/g, the number of viable cells only decreased by approximately 90% at the end of sausage processing.

Clostridial species (Table 4)

Christiansen et al. (*21*) studied the effect of nitrite on *C. botulinum* spores added to summer style sausage prepared with and without starter culture (*L. plantarum* plus *P. cerevisiae*). At nitrite levels of 50 to 150 ppm, the starter culture plus glucose (2%) prevented toxin formation by *C. botulinum*. In the absence of glucose, the starter culture was not effective. Toxic sausages were not found when glucose was present whether or not a starter culture was used. Sausages contained botulinum toxin when both glucose and starter culture were omitted or when nitrite was not used (*21*). These workers demonstrated that the pH drop resulting from fermentation of glucose was the single most important factor in controlling botulinum toxin formation in fermented sausages. Addition of active starter cultures offered an advantage in that the pH decreased more rapidly as compared with allowing the indigenous flora to ferment the sugar.

Vacuum-packed bacon treated with 120 ppm nitrite was not protected against the formation of botulinum toxin for

TABLE 3. *Investigations on the effect of meat starter cultures on the growth and survival of salmonellae present in meat products.*

Foodborne pathogen (food)	Starter culture organisms	Reference
Salmonella dublin (pepperoni)	*Lactobacillus plantarum* plus *Pediococcus cerevisiae*	*81*
S. dublin (Lebanon bologna)	*L. plantarum* plus *P. cerevisiae*	*82*
S. typhimurium (cooked mechanically deboned poultry meat)	*L. plantarum* plus *P. cerevisiae*	*68*
S. typhimurium (pepperoni)	*L. plantarum* plus *P. cerevisiae*	*81*
S. typhimurium (Lebanon bologna)	*L. plantarum* plus *P. cerevisiae*	*82*
S. typhimurium (summer sausage)	*L. plantarum*	*54*
S. senftenberg (dry fermented turkey sausage)	*P. cerevisiae*	*10*
S. senftenberg (Rohwurst, a dried fermented sausage)	a *Lactobacillus* species plus a *Micrococcus* species	*76*
S. newport (summer sausage)	*L. plantarum*	*54*
S. pullorum (dry fermented turkey sausage)	*P. cerevisiae*	*10*

an extended period if sucrose was omitted from the cure (*84*). *L. plantarum* plus ≥0.5% sucrose gave extended protection against *C. botulinum* in bacon. In the absence of starter culture, sucrose plus nitrite gave variable results. Since acid production by the lactic acid bacteria-sucrose combination protected bacon against *C. botulinum* in the absence of nitrite, it was possible to lower the concentration of nitrite in bacon and still prevent production of botulinum toxin (*84*).

Addition of radiation-killed *P. cerevisiae* prevented formation of toxin in *C. botulinum*-contaminated canned commercial ham upon tempertaure abuse (*51*). The pediococci were unable to reproduce (killed) but were still capable of producing acid from fermentable sugars. Sugars (usually sucrose) are added to many processed meat products. Addition of radiation-killed *P. cerevisiae* did not produce any change in the product as long as refrigeration was maintained, but upon temperature abuse, the cells formed acid from the sugar. The resulting decrease in pH inhibited growth and toxin formation by *C. botulinum* (*51*).

Baran and Stevenson (*10*) studied the fate of *Clostridium perfringens* during fermentation and subsequent drying of fermented turkey sausage using *P. cerevisiae* as the starter culture. They showed that *C. perfringens* did not grow and the numbers of viable cells decreased during processing. However, a small population of *C. perfringens* persisted in the final product. It is difficult to determine if the decrease in *C. perfringens* population depended on a lowered pH brought about by the starter culture (the final pH of the sausages was not given), or was due to drying or to a combination of the two processes. In general, the acid conditions found in fermented sausages appear to limit growth and toxin production by clostridial species.

Staphylcocci (Table 5)

Meat products have often been implicated in staphylococcal food poisoning outbreaks. Several outbreaks have occurred when staphylococcal enterotoxin-containing fermented sausages were consumed (*6,19*). A number of investigators have studied growth and survival of *Staphylococcus aureus* during the processing of a variety of fermented sausages. At a ratio of *P. cerevisiae* to *S. aureus* of 10^6:10^1, Barber and Deibel (*12*) found a limited amount of staphylococcal growth at the surface of summer sausages, but no staphylococci were found in the core of the sausages. Baran and Stevenson (*10*) also found that the numbers of *S. aureus* increased during processing of a dry fermented turkey sausage when *P. cerevisiae* was used as the starter culture. The final pH of the summer sausage and turkey sausage were not given.

Daly et al. (*25*) thoroughly studied the effect of three different starter cultures on growth of *S. aureus* in beaker sausage (sausage formulation packed tightly in 50 to 100-ml beaker, and covered with foil) containing a summer sausage formulation (Table 6). Starter cultures of *P. cerevisiae*, *L. plantarum*, or a mixture of the two lactic acid

TABLE 4. *Investigations on the effect of meat starter cultures on the growth and survival of clostridial species present in meat products.*

Foodborne pathogen (food)	Starter culture organisms	Reference
Clostridium botulinum (summer-style sausage)	*Lactobacillus plantarum* plus *Pediococcus cerevisiae*	*21*
C. botulinum (bacon)	*L. plantarum*	*84*
C. botulinum (canned ham)	*P. cerevisiae* (radiation-killed)	*51*
C. perfringens (dry fermented turkey sausage)	*P. cerevisiae*	*10*

TABLE 5. *Investigations on the effect of meat starter cultures on the growth and survival of staphylococci in meat products.*

Foodborne pathogens (food)	Starter culture organisms	Reference
Staphylococcus aureus (summer sausage)	*Pediococcus cerevisiae*	*12*
S. aureus (summer sausage-beaker sausage)	1) *P. cerevisiae* or 2) *Lactobacillus plantarum* or 3) *L. plantarum* plus *P. cerevisiae*	*25*
S. aureus (dry fermented turkey sausage)	*P. cerevisiae*	*10*
S. aureus (Dutch dry fermented sausage)	a *Lactobacillus* species	*50*
S. aureus (dry fermented sausage)	a *Micrococcus* species plus a *Lactobacillus* species	*62*
S. aureus (fermented beef sausage)	*P. cerevisiae* plus *L. plantarum*	*78*
S. aureus (cooked mechanically deboned Poultry meat)	1) *P. cerevisiae* or 2) *L. plantarum* or 3) *L. plantarum* plus *P. cerevisiae*	*68*
S. aureus (Italian dry salami)	a *Lactobacillus* species	*55,56*
S. aureus (Genoa and pepperoni)	*P. pentosaceum*	*66*
S. aureus (commercial canned ham, vacuum-packed smoked turkey, and vacuum-packed ground beef)	*P. cerevisiae* (radiation killed)	*51*

bacteria effectively limited the growth of *S. aureus* at 37°C when the initial level of the pathogen was 10^4/g. The starter cultures did not prevent growth of *S. aureus* at 30 or 21°C (Table 6). They attributed the growth of staphylococci at the lower temperatures to the slower acid production by the starter cultures. Daly and his coworkers (*25*) also found that starter cultures could not prevent growth of *S. aureus* at 37°C if the initial inoculum of staphylococci was raised from 10^4 to 10^5/g even though the final pH of the sausages reached 4.2 to 4.3. Thus starter cultures were not effective in inhibiting *S. aureus* growth if the inoculum of staphylococci was high or if the temperature of fermentation was low. Results obtained with beaker sausage, however, may not be comparable to those obtained with regular sausage.

Labots (*50*) suggested that nitrite was the most important factor in controlling *S. aureus* growth in Dutch fermented sausages, and that the decrease in pH induced by starter cultures played a minor role if sufficient nitrite (120 ppm) was present. Enterotoxin A was not formed in sausages containing a starter culture consisting of a *Lactobacillus-Micrococcus* combination (*62*). Whether starter culture was present or not, enterotoxin B was not detected; however, enterotoxin C was detected in sausage prepared with starter culture but did not persist in the final product. Niskanen and Nurmi (*62*) suggested that the *Micrococcus* species was responsible for inhibition of enterotoxin production.

The destruction of *S. aureus* in fermented sausages was closely related to glucose level and to the rate of lactic acid production by *L. plantarum*/-*P. cerevisiae* starter culture (*78*). Using 2% glucose and an initial *S. aureus* inoculum of 10^7/g, no viable staphylococci were detected at 60 h at 37°C; however, with 0.5% glucose, decrease in the *S. aureus* population to undetectable levels required 130 h.

Genoa salami and pepperoni prepared with *P. pentosaceus* as the starter culture supported limited growth of *S. aureus* (one log or less when the initial count was 10^4/g) at the outer surface of the sausages at temperatures ranging from 21 to 35°C (*66*). Staphylococcal counts of 10^5/g or less would not be expected to result in enterotoxin production; Barber and Deibel (*12*) have shown that counts must be 10^7 to 10^8/g for detectable enterotoxin production. In temperature-abused commercial canned ham, vacuum-packed smoked turkey or ground beef, Lee et al. (*51*) have shown that radiation-killed *P. cerevisiae* added to the foods will produce acid from the sugar present, and thus inhibit growth and enterotoxin production by *S. aureus*.

The studies on the effect of the lactic acid starter cultures on foodborne pathogens indicate that starters effectively limit pathogen growth in general. The inhibitory effects of the starter cultures depends on their ability to form lactic acid rapidly from fermentable sugars added to meats.

CONCLUSIONS

Use of starter cultures to produce fermented meat products is desirable for a number of reasons. Lactic acid starter cultures with known metabolic activity provide lactic acid quickly and consistently (a fermentable sugar must be provided). Processing times are reduced because it is not necessary to select and grow (approximately 15-20 h) the desired microbial species by time-consuming aging of salted meat or by using "back-slopping" techniques. Thus large inventories of raw materials are not needed, and there is less handling of raw meats. Batch-to-batch variation in the product is decreased because starter cultures are consistent in their acid production. This, in turn, results in products with more consistent quality, flavor, and overall desirability. Fermentation failures due to spoilage microorganisms overwhelming the natural flora lactic acid bacteria are reduced or eliminated because of the massive inoculum added to the meat. A very significant advantage to the accelerated acid production by starter cultures is the inhibition of foodborne pathogens and/or microbial toxin production as well as spoilage organisms. Likewise, the possible build-up of pathogens due to the use of "back-slopping" techniques is prevented. The appropriate use of starter cultures appears to offer clear advantages for increasing the quality, productivity, and safety of fermented sausage production.

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TABLE 6. *Effect of lactic acid starter cultures on growth of* S. aureus *in beaker sausage (modified from ref. 25).*

Microorganisms	*S. aureus* count/g after 50 h at		
	21°C	30°C	37°C
S. aureus[a]	7.1×10^7 (5.7)[b]	5.8×10^7 (5.3)	6.3×10^7 (5.2)
S. aureus plus *P. cerevisiae*	2.0×10^6 (4.8)	2.2×10^5 (4.5)	9.0×10^4 (4.3)
S. aureus plus *L. plantarum*	1.8×10^6 (4.7)	5.3×10^5 (4.4)	4.0×10^4 (4.3)
S. aureus plus *L. plantarum* and *P. cerevisiae*	3.1×10^6 (4.7)	5.9×10^5 (4.5)	4.0×10^4 (4.2)

[a]The initial count of *S. aureus* was 3.0×10^4/g; that of the lactic acid bacteria was approximately 10^8 total cells/g.
[b]Numbers in parentheses are final pH values of the beaker sausage; the initial pH of the sausage ranged from 5.9 to 6.0

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